

*AMESON MICHAELIS* (MICROSPORIDA) IN THE BLUE CRAB,  
*CALLINECTES SAPIDUS*: PARASITE-INDUCED ALTERATIONS IN  
THE BIOCHEMICAL COMPOSITION OF HOST TISSUES

A. M. FINDLEY,<sup>1</sup> E. W. BLAKENEY, JR., AND E. H. WEIDNER

*Department of Zoology & Physiology, and Department of Biochemistry, Louisiana State University,  
Baton Rouge, Louisiana 70803*

ABSTRACT

*Ameson michaelis*' interaction with its blue crab host significantly altered the biochemical constituents of host tissues. Modifications in skeletal muscle protein and carbohydrate metabolism were reflected in substantial variations in hemolymph composition. Blood osmolality,  $Cl^-$ , and  $Na^+$  ion levels decreased with heavy parasitic invasion, while  $K^+$  ion and ninhydrin positive substances (NPS) levels increased in both light and heavy infections. Microsporidiosis resulted in a general increase in all hemolymph free amino acids detected except glutamic acid.

Effects of *A. michaelis* sporogenesis were observed by comparing the biochemical composition of thoracic and cheliped skeletal musculature. Protein and carbohydrate levels were lower in infected thoracic muscle. The opposite trend was observed for tissue free-amino-acids (ninhydrin-positive substances). The concentration of nine of the sixteen amino acids detected remained unchanged with infection. Skeletal muscle glutamate, proline, glycine, alanine, and arginine levels declined, while taurine and tyrosine levels increased.

Lactic acid accumulated in the hemolymph, thoracic muscle, and hepatopancreas of parasitized blue crabs. Lactate concentrations reached six to seven times their normal levels in hemolymph and skeletal muscle, and four times the control value in the hepatopancreas. Blood glucose levels declined during the terminal stages of microsporidan infection.

INTRODUCTION

The Microsporidae are a large group of highly specialized obligate intracellular protozoan parasites. Although they are best known as parasites of arthropods and fish, microsporidians are the etiological agents of various pathologies in amphibians, reptiles, birds, and a variety of mammals, including man (see Sinderman, 1970; Weiser, 1976; Canning, 1977). Despite these parasites' wide distribution in nature and their devastating effects on a variety of animals, relatively little is known about their physiological characteristics and the infections they produce.

Disturbances in the biochemical composition of tissues infected by intracellular parasites are of interest because such infections often significantly alter the electrolyte, carbohydrate, protein, and free-amino-acid pools of host cells (see von Brand, 1973). Among parasitic protozoans, the microsporidan *Ameson michaelis* (= *Nosema michaelis*) massively invades host cells, proceeds rapidly through sporogenesis in host muscle, and inflicts widespread tissue damage. *A. michaelis* sporogenesis takes place in blue crab sarcoplasm. After entering myofibrils, schizonts

Received 12 March 1981; accepted 1 June 1981.

<sup>1</sup> Present address: Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803

differentiate into sporonts, and then multiply into sporoblasts (Weidner, 1970). The massive numbers of sporoblast colonies in the sarcoplasm result in the disorientation and eventual loss of the highly organized myofibrillar structure of the host muscle (Weidner, 1970). As the infection proceeds to its terminal stages, parasite spores largely replace the host skeletal musculature (up to  $10^9$  parasites/g infected muscle).

The present study characterizes the physiological consequences of microsporidian infection in blue crab skeletal muscle, hemolymph, and hepatopancreas tissues.

#### MATERIALS AND METHODS

Adult intermolt blue crabs (*Callinectes sapidus*), uninfected and infected with *Ameson michaelis*, were collected from Lake Pontchartrain near Irish Bayou, Louisiana, during August and September, 1978 and 1979. Animals were transported to the laboratory over ice and then killed immediately. Small sections of thoracic skeletal muscle tissue were examined microscopically to determine the extent of parasite development in individual animals. Blue crabs exhibiting  $\leq 10^3$  parasites/g tissue were considered lightly infected;  $> 10^3$  parasites/g tissue was considered a heavy infection.

##### *Hemolymph analyses*

*Osmolality and ion analyses:* The cuticle at the base of the pereiopod (swimming leg) was blotted dry and a 300–400  $\mu$ l sample of hemolymph was withdrawn into snap-cap microcentrifuge tubes using unheparinized capillary tubes. Blood samples were centrifuged immediately at 10,000 g for 4 min to remove any particulate material. The osmolality of hemolymph and seawater samples was determined with a WESCOR Vapor Pressure Osmometer. Hemolymph samples were diluted with deionized water for ion analyses. Chloride ion concentrations were determined with an Aminco Chloridometer. Sodium and potassium levels were determined with a Coleman Flame Photometer.

*Ninhydrin positive substances:* Additional hemolymph samples were collected to determine ninhydrin positive substances (NPS). Protein was precipitated from the hemolymph by diluting each sample with 5% sulfosalicylic acid to a final concentration of 10%. Following centrifugation (10,000 g; 4 min), the NPS level of the supernatant was determined colorimetrically by the method of Rosen (1957). Leucine was used as the ninhydrin standard. Amino acid composition of the protein-free supernatant was determined with a Beckman Amino Acid Analyzer, Model 119.

*Glucose analyses:* Hemolymph samples (0.2 ml HL in 2.0 ml distilled water) were deproteinized by adding 1.0 ml 0.3 N Ba(OH)<sub>2</sub> and 1.0 ml 5% ZnSO<sub>4</sub>·7H<sub>2</sub>O. Following centrifugation (10,000 g; 4 min), the glucose concentration of the protein-free supernatant was quantified colorimetrically by the glucose oxidase-peroxidase method (Sigma Chemical Co., Kit No. 510).

*Lactate analyses:* Immediately after they were collected, 0.2 ml hemolymph samples were transferred into microcentrifuge tubes containing 0.4 ml cold 8% perchloric acid. The tubes were agitated and returned to an ice bath for 5 min to assure complete precipitation of blood proteins. The mixture was subsequently centrifuged (10,000 g; 15 min) and the lactate concentration of the protein-free supernatant was quantified enzymatically (Sigma Chemical Co., Kit No. 826–UV).

### *Skeletal muscle and hepatopancreas analyses*

**Protein, NPS, and carbohydrate concentrations:** Skeletal muscle tissue was removed from the thoracic region and the pereopods (including the chela, walking legs, and paddles). Dissected tissue was flash frozen over dry ice, lyophilized to dryness, and ground to a uniform particle size in a Thomas-Wiley tissue grinder. Ground tissue was stored frozen ( $-20^{\circ}\text{C}$ ) in 1-dram glass vials with cork stoppers. When it was needed, ground tissue was removed from the freezer and warmed to room temperature in a dessicator to prevent water adsorption. Protein was extracted by refluxing 10 mg of the ground tissue in 1 *N* NaOH for 30 min at  $100^{\circ}\text{C}$ . The protein content of the tissue extracts was determined by the method of Lowry *et al.* (1951). Bovine serum albumin was used as the reference standard. Additional portions of the NaOH extracts were analyzed for carbohydrate concentration (Montgomery, 1957), using glycogen as the standard. Hepatopancreas samples were similarly prepared and analyzed for their protein and carbohydrate content.

To measure NPS in tissue, 10 mg of ground skeletal muscle tissue was incubated in 10% 5-sulfosalicylic acid (48 h;  $22^{\circ}\text{C}$ ) to precipitate proteins and permit leaching of free amino acids from the tissue. Samples were then centrifuged (10,000 g; 15 min) and supernatant NPS was determined (Rosen, 1957), using leucine as the standard. Amino acid composition of the NPS digest was determined with a Beckman Amino Acid Analyzer, Model 119.

**Lactate concentrations:** Samples of thoracic skeletal muscle and hepatopancreas were removed, flash frozen over dry ice, and weighed. Individual samples were homogenized in 10 times their weight of cold 8% perchloric acid. Homogenates were transferred to microcentrifuge tubes and returned to an ice bath for 5 min to allow precipitation of tissue proteins. Samples were centrifuged (10,000 g; 15 min) and the lactate concentration of the protein-free supernatant was quantified enzymatically (Sigma Chemical Co., Kit No. 826-UV).

## RESULTS

### *Hemolymph profile*

Salinity at the time crabs were collected was 2–6‰ S, water temperature varied from  $29$ – $34^{\circ}\text{C}$ , and the dissolved oxygen content ranged between 5–7 mg/l. Water under these temperature and salinity conditions was 70–95% saturated with dissolved oxygen.

During both years, *A. michaelis* infection markedly altered blue crab hemolymph composition. Blood osmolality and  $\text{Cl}^{-}$  and  $\text{Na}^{+}$  ion levels decreased with infection, while  $\text{K}^{+}$  ion and ninhydrin positive substances (NPS) levels increased (Table I). Light infection produced little change in hemolymph osmolality,  $\text{Na}^{+}$ , and  $\text{Cl}^{-}$  ion concentrations ( $P > 0.05$ ), whereas blood  $\text{K}^{+}$  values were 25% ( $\sim 1.5$ – $2.0$  mM/l) higher, and NPS levels were nearly twice (or approximately 3–4 mM/l higher than) those of uninfected animals ( $P < 0.05$ ).

A heavy parasite burden was accompanied by significant modifications in all blood constituents assayed ( $P < 0.01$ ). Hemolymph osmolality decreased by 10% ( $\sim 60$ – $70$  mOsm/kg water), and  $\text{Cl}^{-}$  and  $\text{N}^{+}$  ion values each declined by 15% (approximately 50 mM/l) of their control levels.  $\text{K}^{+}$  ion concentrations were 60% or nearly 5 mM/l higher, and NPS values 2.5–3 times or approximately 8 mM/l higher, than those of control animals.

The amino acid composition of hemolymph from infected blue crabs was substantially different from that of controls (Table II). *A. michaelis* infection increased

TABLE I

*Effects of microsporidan infection on the hemolymph composition of blue crabs. Values are for blue crabs collected from Lake Pontchartrain, Louisiana, during the summer of 1978 (top line of each entry) and 1979 (bottom line of each entry). Salinity at the time of collection was 2-4‰ S (1978) and 2-6‰ S (1979). All hemolymph values are the mean ± 95% confidence interval; sample size in parentheses.*

	Normal	Infected	
		Light	Heavy
Osmolality (mOsm/kg H <sub>2</sub> O)	663.3 ± 17.5 (10) 671.6 ± 21.5 (5)	641.3 ± 6.7 (10) 639.5 ± 10.1 (5)	594.2 ± 10.4 (10) 594.3 ± 9.9 (5)
Cl <sup>-</sup> mM/l	327.7 ± 8.1 (10) 328.0 ± 1.7 (5)	314.5 ± 13.6 (10) 317.0 ± 5.4 (5)	278.5 ± 8.6 (10) 275.0 ± 10.5 (5)
Na <sup>+</sup> mM/l	304.7 ± 8.6 (10) 304.3 ± 6.8 (5)	290.5 ± 5.9 (10) 294.7 ± 6.8 (5)	259.0 ± 13.0 (10) 261.0 ± 12.5 (5)
K <sup>+</sup> mM/l	7.6 ± 0.8 (10) 8.2 ± 0.5 (5)	9.7 ± 0.4 (10) 9.9 ± 0.5 (5)	12.2 ± 1.5 (10) 13.3 ± 1.1 (5)
NPS mM/l	4.1 ± 0.8 (10) 5.4 ± 0.6 (5)	8.3 ± 0.6 (10) 8.3 ± 1.1 (5)	12.4 ± 1.9 (10) 12.6 ± 2.4 (5)

the hemolymph concentration of ammonia and seven of the nine amino acids detected. Infected animals exhibited a 50% increase in hemolymph glycine concentration, a two- or three-fold increase in arginine, aspartic acid, taurine, threonine, and NH<sub>3</sub> levels, and a seven- or eight-fold increase in alanine and proline concentrations. Glutamic acid decreased 60%, and was the only amino acid to decline in concentration in infected animals.

### *Skeletal muscle profile*

Microsporidan infection significantly changed host concentrations of muscle protein, free amino acids (NPS), and carbohydrates. However, since the level of these biochemical constituents varied widely among different blue crabs, data were

TABLE II

*Amino acid composition of normal and infected blue crab hemolymph. All amino acid values are the mean ± 95% confidence interval; sample size in parentheses. Significance refers to t test comparisons between normal and infected values. NS means not significant at P < 0.05 level.*

μmoles/ml	Normal (N = 4)	Infected (N = 6)	Significance
tau	0.380 ± 0.160	1.060 ± 0.264	P < 0.01
asp	0.156 ± 0.005	0.357 ± 0.079	P < 0.05
thr	0.027 ± 0.011	0.087 ± 0.021	P < 0.05
ser	0.084 ± 0.014	0.118 ± 0.041	NS
glu	0.200 ± 0.013	0.117 ± 0.022	P < 0.01
pro	0.071 ± 0.010	0.544 ± 0.129	P < 0.01
gly	0.767 ± 0.108	1.176 ± 0.293	P < 0.05
ala	0.074 ± 0.008	0.475 ± 0.141	P < 0.01
arg	0.162 ± 0.018	0.518 ± 0.049	P < 0.01
NH <sub>3</sub>	3.235 ± 0.681	5.947 ± 0.566	P < 0.01

analyzed as the percent difference between the thoracic and cheliped muscle value for each constituent in single animals. Comparing thoracic and cheliped muscle values maximized the detection of infection-related changes in biochemical composition, because the thoracic region is the first and most heavily infected of the crab skeletal musculature, while the claw tissue, apparently secondarily infected, often contains few parasites until the terminal stages of the infection.

Skeletal muscle protein varied between 600–750  $\mu\text{g}/\text{mg}$  dry tissue in all animals sampled. Light and heavy infections resulted in significantly lower thoracic than cheliped muscle protein (Table III). Skeletal muscle free-amino-acid levels ranged from 0.8–1.3  $\mu\text{moles}/\text{mg}$  dry tissue. In infected animals, thoracic muscle NPS concentrations were significantly higher than cheliped levels (Table III). Heavy infections produced substantially greater alterations in the protein and NPS ratio of thoracic and cheliped muscle than did lighter parasite burdens ( $P < 0.01$ ).

The amino acid composition of infected thoracic muscle differed from that of uninfected tissue (Table IV). Infected thoracic muscle contained reduced quantities of alanine, arginine, glutamic acid, glycine, and proline. Arginine levels dropped  $\sim 65\%$ , alanine and proline values declined  $\sim 50\%$ , and glutamic acid and glycine decreased approximately 30% in concentration. Infected muscle taurine and tyrosine values were twice those of normal animals. Muscle aspartic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, and valine concentrations remained the same as their control values ( $P > 0.05$ ). Tissue  $\text{NH}_3$  levels also did not change with infection ( $P > 0.05$ ).

Skeletal muscle carbohydrate concentration varied from 20–35  $\mu\text{g}/\text{mg}$  dry tissue in all animal groups. Both light and heavy infections produced significantly lower thoracic than cheliped muscle carbohydrate levels ( $P < 0.01$ ) (Table III). The conditions in infected animals also differed statistically from the control condition ( $P < 0.01$ ).

Parasitic infection produced no significant differences in hepatopancreas carbohydrate (from  $15.9 \pm 0.5$  to  $15.2 \pm 0.9$   $\mu\text{g}/\text{mg}$  dry tissue,  $P > 0.05$ ) or protein (from  $250.0 \pm 11.7$  to  $223.0 \pm 10.4$   $\mu\text{g}/\text{mg}$  dry tissue,  $P > 0.05$ ) levels.

### Lactate levels

Microsporidan infection substantially increased lactic acid levels in hemolymph, skeletal muscle, and hepatopancreas host tissues (Table V). Hemolymph samples had the lowest control lactic-acid levels. Light infections produced a fourfold in-

TABLE III

*Effect of microsporidan infection on the skeletal muscle composition of blue crabs. Data are the percent difference between thoracic and cheliped muscle levels of protein, free amino acid (NPS), and carbohydrates. Arrows indicate whether values for thoracic muscle were higher ( $\uparrow$ ) or lower ( $\downarrow$ ) than those of cheliped muscle. All values are the mean  $\pm$  95% confidence interval; sample size in parentheses.*

	Normal (N = 6)	Infected	
		Light (N = 10)	Heavy (N = 6)
Protein	$10.97 \pm 0.24$	$\downarrow 5.98 \pm 1.18$	$\uparrow 12.45 \pm 0.65$
NPS	$\uparrow 2.60 \pm 0.93$	$\uparrow 11.49 \pm 3.46$	$\uparrow 27.03 \pm 5.45$
Carbohydrate	$\uparrow 6.00 \pm 0.71$	$\downarrow 4.65 \pm 1.21$	$\uparrow 18.07 \pm 3.04$

TABLE IV

*Amino acid profile of thoracic skeletal muscle from normal and infected blue crabs. All amino acid values are the mean  $\pm$  95% confidence interval; sample size in parentheses. Significance refers to *t* test comparisons between values. NS means not significant at the  $P < 0.05$  level.*

$\mu$ moles/mg	Normal (N = 4)	Infected (N = 6)	Significance
tau	0.096 $\pm$ 0.019	0.193 $\pm$ 0.038	$P < 0.05$
asp	0.029 $\pm$ 0.003	0.029 $\pm$ 0.003	NS
ser	0.043 $\pm$ 0.008	0.044 $\pm$ 0.015	NS
glu	0.095 $\pm$ 0.011	0.061 $\pm$ 0.012	$P < 0.05$
pro	0.189 $\pm$ 0.027	0.095 $\pm$ 0.034	$P < 0.05$
gly	0.508 $\pm$ 0.049	0.352 $\pm$ 0.053	$P < 0.05$
ala	0.192 $\pm$ 0.032	0.106 $\pm$ 0.027	$P < 0.05$
val	0.015 $\pm$ 0.001	0.014 $\pm$ 0.002	NS
met	0.011 $\pm$ 0.004	0.010 $\pm$ 0.004	NS
ile	0.009 $\pm$ 0.004	0.010 $\pm$ 0.004	NS
leu	0.030 $\pm$ 0.023	0.016 $\pm$ 0.009	NS
tyr	0.011 $\pm$ 0.001	0.022 $\pm$ 0.003	$P < 0.05$
phe	0.008 $\pm$ 0.001	0.009 $\pm$ 0.004	NS
his	0.017 $\pm$ 0.004	0.015 $\pm$ 0.005	NS
lys	0.021 $\pm$ 0.003	0.021 $\pm$ 0.007	NS
arg	0.319 $\pm$ 0.037	0.099 $\pm$ 0.030	$P < 0.01$
NH <sub>3</sub>	0.116 $\pm$ 0.022	0.130 $\pm$ 0.028	NS

crease and heavy infections generated a sevenfold increase in crab hemolymph lactate concentration ( $P < 0.01$ ). Light parasite burdens doubled-thoracic muscle lactate ( $P < 0.01$ ), whereas heavy infections yielded a sixfold increase in muscle lactate ( $P < 0.01$ ). Control lactate values were higher for the hepatopancreas than for either of the other normal tissues. A 65% elevation of hepatopancreas lactate concentrations accompanied light infections ( $P < 0.05$ ), while heavy infections produced a 4.5-fold increase in normal lactate levels ( $P < 0.01$ ).

Blue crabs also evidenced hypoglycemia in the terminal stages of *A. michaelis* infection. Hemolymph glucose levels declined from a control value of  $66.3 \pm 2.8$  mg/100 ml to  $51.1 \pm 3.5$  mg/100 ml. This was significant difference ( $P < 0.01$ ) between normal and infected animals.

## DISCUSSION

Little of the considerable literature pertaining to the Microsporida deals with physiological changes in host animals as a result of microsporidan infection. During

TABLE V

*Lactate concentration in the hemolymph, thoracic skeletal muscle and hepatopancreas of blue crabs. All values are the mean  $\pm$  the 95% confidence interval; sample size in parentheses.*

	Normal (N = 6)	Infected	
		Light (N = 6)	Heavy (N = 6)
Hemolymph mg/ml	0.110 $\pm$ 0.050	0.441 $\pm$ 0.036	0.722 $\pm$ 0.032
Thoracic muscle mg/g	0.228 $\pm$ 0.051	0.548 $\pm$ 0.022	1.237 $\pm$ 0.276
Hepatopancreas mg/g	0.314 $\pm$ 0.069	0.512 $\pm$ 0.062	1.465 $\pm$ 0.322

infection, the host cell environment may influence the metabolic activities of an intracellular parasite. In the present study, we have shown that the interactions of the microsporidan parasite *Ameson michaelis* with its blue crab host, *Callinectes sapidus*, result in thoracic skeletal muscle alterations that are ultimately reflected in biochemical changes in the host blood.

The estuarine environment of the blue crab experiences rapid fluctuations in osmotic and ionic composition. *Callinectes sapidus* has considerable capacity for osmotic and ionic regulation over widely fluctuating salinity regimens (Findley and Stickle, 1978). This capacity for water and salt regulation varies with a number of exogenous and endogenous factors, among the latter parasitism. *A. michaelis* appears to infect blue crabs most readily in areas of low salinity, high temperature, and reduced oxygen (personal observation). The same combination of factors also stresses the crabs' regulatory abilities (Kinne, 1971).

The hemolymph composition of control crabs used in this study was consistent with that previously described for blue crabs maintained at extremely low salinities (Mangum and Amende, 1972; Cameron, 1978). As a consequence of parasitic infection, hemolymph osmotic and ionic composition changed significantly. Blood osmolality,  $\text{Cl}^-$ , and  $\text{Na}^+$  ion concentrations decreased with infection, while  $\text{K}^+$  ion and free amino acid (NPS) levels increased. This may result in part from the release of ions and free amino acids accompanying host cell lysis. However, cell lysis cannot account for the drop in blood osmolality during heavy infection, because the extracellular and intracellular fluid compartments are isosmotic. Blood composition was significantly changed primarily during heavy microsporidan infection. Severe parasite invasion results in the production of large numbers of mature spores and concomitant disruption of host skeletal muscle tissue (Weidner, 1970). Animals supporting light infections (with proportionally larger numbers of young sporoblast stages present) did not have substantially altered hemolymph profiles. Presumably, during light infection, blue crabs are able to accommodate initial host-cell lysis. However, their regulatory abilities may become inadequate to handle the extensive cell damage that occurs during the terminal stages of infection.

In contrast to our data, Vivarès and Cuq (1981) observed no change in hemolymph ion content of crabs (*Carcinus mediterraneus*) parasitized by the microsporidan *Thelohania maenadis*. However, the differing environmental regimens to which the two species of crab were exposed (2–6‰ S; 29–34°C for *Callinectes sapidus* vs. 33‰ S; 18°C for *Carcinus mediterraneus*) may explain these conflicting results, and consequently, the relative importance of the host blood ion composition during parasitic infection. *C. sapidus* maintained at extremely low salinities must regulate its hemolymph hyperosmotic and hyperionic to the dilute media. Elevated water temperature and reduced oxygen further aggravates this situation. Conversely, *C. mediterraneus* held at higher salinities (generally isosmotic and isonic to the crab blood) does not have water and salt regulation as limiting factors for survival.

*A. michaelis* infections also substantially altered the hemolymph free-amino-acid profile. In salinities of 16.5–21.0‰ S, glycine, taurine, alanine, proline, and arginine constitute 72–90% of the total free amino acids in the serum of mature *Callinectes sapidus* (Lynch and Webb, 1973a). At extremely low salinities (2–4‰ S), we detected only nine amino acids and ammonia in appreciable quantities. The relative concentrations of proline and alanine appeared to be reduced, while the levels of glutamic and aspartic acids were proportionally increased.

In the blue crab, microsporidan infection contributed to a general elevation of all blood free amino acids except glutamic acid. These increases may be primarily

drived from host muscle proteolysis, but parasite invasion may also modify the permeability of muscle cell membranes, so that intracellular amino acids leak to the extracellular spaces. Such a mechanism operates in osmotic and volume regulation in euryhaline animals (Fugelli, 1967; Vincent-Marique and Gilles, 1970). Accumulation of amino acids in the body fluids of infected animals may indicate that their capacity to hydrolyze host cell protein is greater than their ability to incorporate the resultant amino acids into parasite protein (Moulder, 1962). Therefore, the decline we observed in hemolymph glutamic acid may indicate its uptake by developing parasites. Similarly, parasites may use those amino acids which increase only moderately in infected animals (*i.e.*, glycine, aspartic acid, and serine). In fact, acid hydrolysates of *Ameson michaelis* spores indicate large quantities of glutamic acid, glycine, aspartic acid, and serine, in addition to significant amounts of tyrosine (Weidner and Blakeney, unpublished data).

Only two studies deal with host blood amino acids during microsporidiosis (Wang and Moeller, 1970; Vivarès *et al.*, 1980). *Nosema apis*-infected honey bees have less hemolymph amino acids than their non-infected counterparts. Wang and Moeller (1970) suggested this decrease reflects the parasite's utilization of the host blood free-amino-acid pool. At first glance, our data, describing increased levels free amino acids in plasma, appear inconsistent with those of Wang and Moeller. However, this discrepancy is probably attributable to the use of animals supporting different stages of microsporidan infection. Five- to ten-day-old infected honey bees contain proportionally larger numbers of younger, more metabolically active vegetative and early spore-forming stages than do blue crabs sustaining terminal infections, represented by the abundance of mature spores. Additionally, honey bees, as insects, have a larger hemolymph free-amino-acid pool ( $\sim 28\text{--}65\text{ mM/l}$ ), and consequently a more substantial amino acid source for developing parasites, than do blue crabs, which have lower blood amino-acid levels ( $\sim 4\text{--}5\text{ mM/l}$  at 2–6‰ S). Vivarès *et al.* (1980) monitored the effect of *Thelohania maenadis* infection on the hemolymph amino acid composition of *Carcinus mediterraneus*. Although parasitism did not substantially change the total free-amino-acid level, the concentration of several amino acids increased moderately (including taurine, cysteic acid, aspartic acid, threonine, serine, glutamic acid, alanine, methionine, histidine, and arginine), while proline and glutamine levels decreased.

*Ameson michaelis* initially invades skeletal muscle in the thoracic region, near the crab midgut submucosa, which supports the parasite's early vegetative development. As sporogenesis proceeds, other skeletal muscle masses (*i.e.*, those of the cheliped and other pereopods) are infected (Weidner, 1970). Comparison of thoracic and cheliped muscle composition reflects the effects of sporogenesis. Significant decreases in tissue protein and concomitant increases in free-amino-acid levels may result from substantial muscle proteolysis accompanying terminal parasite development. Additionally, modified membrane permeability may change the relative abundance of muscle, and consequently, hemolymph free-amino-acids (Schoffeniels, 1976). Reduced muscle carbohydrate reserves (*i.e.*, glycogen) may result from increased metabolic demands of host muscle and/or developing parasites.

Low salinity significantly reduced the crab-muscle free-amino-acid pool ( $\sim 0.8\text{--}1.3\ \mu\text{moles/mg}$  dry tissue, 2–4‰ S, present study, vs.  $\sim 28\ \mu\text{moles/100 mg}$  wet weight, 50% SW and  $\sim 42\ \mu\text{moles/100 mg}$  wet weight, 100% SW, Schoffeniels, 1976). Blue crabs transferred from full-strength to 50% seawater display significant decreases in virtually all muscle free amino acids (Schoffeniels, 1976). At even lower salinities, we observed further reductions in amino acid levels, and in particular, in alanine, glycine, proline, and taurine concentrations. In parasitized tho-



racic skeletal muscle, the concentration of nine of the sixteen amino acids detected was not significantly different. Glutamic acid, proline, glycine, alanine, and arginine levels were lower with infection, while taurine and tyrosine values were higher. Modifications in infected-muscle free-amino-acid levels, with the exception of tyrosine, were reflected in significant changes in plasma concentration. Tyrosine was virtually absent from the hemolymph of low salinity animals but was present in substantial amounts in the acid hydrolysate of *A. michaelis* spores (Weidner and Blakeney, unpublished data). Efficient uptake of tyrosine from the muscle free-amino-acid pool by developing parasites may preclude its measurable accumulation in crab blood.

Microsporidiosis in *Carcinus mediterraneus* only slightly changed the total free-amino-acid concentration of infected skeletal muscle (Vivarès *et al.*, 1980). Increases in the level of several amino acids (taurine, cysteic acid, serine, glutamic acid, alanine, methionine, leucine, tyrosine, phenylalanine, lysine, and histidine) were offset by reductions in other amino acids (threonine, proline, glycine, and arginine). Direct comparison of our results with those of Vivarès *et al.* (1980) is limited by the lack of any statistical treatment of the amino acid data in the latter study. In addition, different environmental salinity-temperature conditions may have changed the relative importance of the host amino-acid pool.

Parasitic infection may result in host tissues being deprived of oxygen (von Brand, 1973). Oxygen insufficiency can result from the increased metabolic demands of host tissues and/or developing parasites, or an impaired oxygen-delivery system. Under these conditions, anaerobic metabolism becomes increasingly important as a means of energy production (Burke, 1979). In crustaceans, glycolysis is a major functional anaerobic pathway. During hypoxia, significant quantities of pyruvate are converted to lactic acid in crustacean skeletal muscle (Dendinger and Schatzlein, 1973; Phillips *et al.*, 1977; Burke, 1979). Non-parasitized blue crabs have hemolymph and thoracic-muscle lactic-acid concentrations similar to those of other crustaceans (Dendinger and Schatzlein, 1973; Burke, 1979). However, lactic-acid concentrations in blue crab hepatopancreas are significantly higher than in other crabs (Dendinger and Schatzlein, 1973). Elevated lactate concentrations may reflect animals collected from a stressed environment (water of low salinity, high temperature, and reduced oxygen) or may be an artifact of handling (Dendinger and Schatzlein, 1973).

*A. michaelis* infection produced significant lactic acid accumulation in the hemolymph, thoracic muscle, and hepatopancreas of parasitized blue crabs. Lactate concentrations reached six–seven times their normal levels in hemolymph and muscle, and four times the control value in the hepatopancreas. Since the amount of lactate was always higher in the thoracic muscle than in the hemolymph, it is reasonable to assume that the muscle is the ultimate source of lactate production (Phillips *et al.*, 1977). The hepatopancreas has both reduced activity of glycolytic enzymes and an insignificant level of *in vitro* lactate production (Dendinger and Schatzlein, 1973; Schatzlein *et al.*, 1973). Therefore, increases in hepatopancreas lactate levels probably represent the clearance of this muscle metabolite from the hemolymph.

Plasma glucose concentrations in freshly collected *Callinectes sapidus* specimens are extremely variable. Our control blood-glucose values were at the high end of the reported normal range (Dean and Vernberg, 1956a; Lynch and Webb, 1973b). Elevated glucose concentrations may be due to handling stress (Telford, 1968), asphyxia (resulting from holding animals out of water) (Lynch and Webb, 1973b), or high temperatures (Dean and Vernberg, 1965b). Parasitized blue crabs

had reduced blood glucose levels during the terminal stages of microsporidan infection (a terminal hypoglycemia). Reduced hemolymph glucose may result from increased metabolic demands of host cells and/or developing parasites.

Lactic acid accumulates in the blood of protozoan-infected animals (von Brand, 1973). However, in whole animal studies, it is difficult to ascertain whether elevated lactate concentrations result from parasite production or host cell metabolism. The relative importance of host cell and parasite carbohydrate metabolism in accumulation of lactate by microsporidan-infected blue crabs cannot be determined directly from the present study.

Microsporidan infection contributed to significant alterations in the biochemical constituents of blue crab host tissues. Modifications in skeletal muscle protein and carbohydrate metabolism were ultimately reflected in substantial changes in hemolymph composition. Characterizing the blue crab host cell environment during *Ameson michaelis* infection provides critical background information for future metabolic studies of isolated, extracellularly maintained microsporidan parasites.

#### ACKNOWLEDGMENTS

Partial funding for this work was provided by NIH grant 1R01 AI 15829-01 to E.H.W. We are grateful to Drs. William B. Stickle and Thomas H. Dietz (Department of Zoology & Physiology, L.S.U.) for their critical review of this manuscript. Our special thanks are extended to Mr. Julian King, commercial fisherman, Irish Bayou, Louisiana, who generously supplied the dozens of healthy and parasitized blue crabs used in this study.

#### LITERATURE CITED

- BURKE, E. M. 1979. Aerobic and anaerobic metabolism during activity and hypoxia in two species of intertidal crabs. *Biol. Bull.* **156**: 157-168.
- CAMERON, J. N. 1978. NaCl balance in the blue crab, *Callinectes sapidus*, in fresh water. *J. Comp. Physiol.* **123**: 127-135.
- CANNING, E. U. 1977. Microsporida. Pp. 155-196 in J. P. Kreier, Ed., *Parasitic protozoa*, Volume IV. Academic Press, New York.
- DEAN, J. M., AND F. J. VERNBERG. 1965a. Variations in the blood glucose of Crustacea. *Comp. Biochem. Physiol.* **14**: 29-34.
- DEAN, J. M., AND F. J. VERNBERG. 1965b. Effects of temperature acclimation on some aspects of carbohydrate metabolism in decapod Crustacea. *Biol. Bull.* **129**: 87-94.
- DENDINGER, J. E., AND F. C. SCHATZLEIN. 1973. Carbohydrate metabolism in the striped shore crab, *Pachygrapsus crassipes*. II. Glycolytic rate of muscle, gill and hepatopancreas. *Comp. Biochem. Physiol.* **45B**: 699-708.
- FINDLEY, A. M., AND W. B. STICKLE. 1978. Effects of salinity fluctuation on hemolymph composition of the blue crab *Callinectes sapidus*. *Mar. Biol.* **46**: 9-15.
- FUGELLI, K. 1967. Regulation of cell volume in flounder (*Pleuronectes flesus*) erythrocytes accompanying a decrease in plasma osmolality. *Comp. Biochem. Physiol.* **22**: 253-260.
- KINNE, O. 1971. Salinity: animals—vertebrates. Pp. 821-995 in O. Kinne, Ed., *Marine biology*, Vol. 1, Part 2. Wiley Interscience, New York.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- LYNCH, M. P., AND K. L. WEBB. 1973a. Variations in serum constituents of the blue crab, *Callinectes sapidus*: free amino acids and total ninhydrin positive substances. *Comp. Biochem. Physiol.* **45B**: 407-418.
- LYNCH, M. P., AND K. L. WEBB. 1973b. Variations in serum constituents of the blue crab, *Callinectes sapidus*: Glucose. *Comp. Biochem. Physiol.* **45A**: 127-139.
- MANGUM, C. P., AND L. M. AMENDE. 1972. Blood osmotic concentration of blue crabs (*Callinectes sapidus* Rathbun) found in fresh water. *Chesapeake Sci.* **13**: 318-320.
- MONTGOMERY, R. 1957. Determination of glycogen. *Arch. Biochem. Biophys.* **67**: 378-386.
- MOULDER, J. 1962. *The biochemistry of intracellular parasitism*. University of Chicago Press, Chicago.

- PHILLIPS, J. W., R. J. W. MCKINNEY, F. J. R. HIRD, AND D. L. MACMILLAN. 1977. Lactic acid formation in crustaceans and the liver function of the midgut gland questioned. *Comp. Biochem. Physiol.* **56B**: 427-433.
- ROSEN, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.* **67**: 10-15.
- SCHATZLEIN, F. C., H. M. CARPENTER, M. R. RODGERS, AND J. L. SUTKO. 1973. Carbohydrate metabolism in the striped shore crab, *Pachygrapsus crassipes*. I. The glycolytic enzymes of gill, hepatopancreas, heart and leg muscles. *Comp. Biochem. Physiol.* **45B**: 393-405.
- SCHOFFENIELS, E. 1976. Biochemical approaches to osmoregulatory processes in Crustacea. Pp. 107-124 in P. S. Davies, Ed., *Perspectives in experimental biology*, Vol. 1. Pergamon Press, New York.
- SINDERMAN, C. J. 1970. *Principal diseases of marine fish and shellfish*. Academic Press, New York.
- TELFORD, M. 1968. The effects of stress on blood sugar composition of the lobster, *Homarus americanus*. *Can. J. Zool.* **46**: 819-826.
- VINCENT-MARIE, C., AND R. GILLES. 1970. Modification of the amino acid pool in blood and muscle of *Eriocheir sinensis* during osmotic stress. *Comp. Biochem. Physiol.* **35**: 479-485.
- VIVARÈS, C. P., J.-L. CUQ, H. J. CECCALDI, AND P. J. RICHARD. 1980. Influence d'une microsporidiose sur les acides aminés libres de *Carcinus mediterraneus* Czerniavsky, 1884 soumis à diverses salinités et à des valeurs extrêmes de température. *J. Exp. Mar. Biol. Ecol.* **43**: 207-220.
- VIVARÈS, C. P., AND J.-L. CUQ. 1981. Physiological and metabolic variations in *Carcinus mediterraneus* (Crustacea:Decapoda) parasitized by *Thelohania maenadis* (Microspora:Microsporida): An ecophysiological approach. *J. Invertebr. Pathol.* **37**: 38-46.
- VON BRAND, T. 1973. *Biochemistry of parasites*. Academic Press, New York.
- WANG, D. I., AND F. E. MOELLER. 1970. Comparison of the free amino acid composition in the hemolymph of healthy and *Nosema*-infected female honey bees. *J. Invertebr. Pathol.* **15**: 202-206.
- WEIDNER, E. 1970. Ultrastructural study of microsporidan development. *Z. Zellforsch.* **105**: 33-54.
- WEISER, J. 1976. Microsporidia in invertebrates: Host-parasite relations at the organismal level. Pp. 163-201 in L. A. Bulla, Jr. and T. C. Cheng, Eds., *Comparative pathobiology*, Vol. 1. Plenum Press, New York.